PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 14187-1PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/CA 03/00547	International filing date *(day/month/year)* 11.04.2003	Priority date *(day/month/year)* 11.04.2003
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant DNA LANDMARKS INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.11.2004	08.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Marinoni, J-C Telephone No. +49 89 2399-8563

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA 03/00547

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-24 as originally filed

Sequence listings part of the description, Pages

1-3 as originally filed

Claims, Numbers

1-39 as originally filed

Drawings, Sheets

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-39
	No:	Claims	none
Inventive step (IS)	Yes:	Claims	1-39
	No:	Claims	none
Industrial applicability (IA)	Yes:	Claims	1-39
	No:	Claims	none

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application relates to a method of assessing an amount of a known target nucleic acid sequence in a sample, said method comprising the steps of:
   a. co-amplifying the target nucleic acid and a known amount of a control sequence, control and target sequence being different, to produce target and control amplicons
   b. determining relative amounts of target and control amplicons by determining realtive quantities of primer extension reactions using the respective amplicons as template,

   wherein the primer extension reaction is performed with sequential addition of the individual nucleotides, such that the primer extension reaction of target and control amplicons are performed sequentially, and wherein determining the relative quantities of primer extenson products comprises comparing the quantity of nucleotides incorporated during each reaction.

2. Reference is made to the following documents:

D1: PIELBERG G ET AL: "Unexpectedly high allelic diversity at the KIT locus causing dominant white color in the domestic pig" GENETICS, vol. 160, no. 1, January 2002 (2002-01), pages 305-311

D2: WO 00/63437, 26 October 2000

D3: WO 02/20837, 14 March 2002

D6: ALDERBORN A ET AL: "Determination of single-nucleotide polymorphisms by real-time pyrophosphate DNA sequencing" GENOME RESEARCH, vol. 10, no. 8, August 2000 , pages 1249-1258

D7: RONAGHI M: "PYROSEQUENCING SHEDS LIGHT ON DNA SEQUENCING" GENOME RESEARCH, vol. 11, no. 1, January 2001, pages 3-11

3. Documents **D1** to **D7** discloses the pyrosequencing method for typing single nucleotide polymorphisms. Although this method is based on the quantification of the nucleotides incorporation, makes use of sequential incorporation of nucleotides, and is based on a primer extension reaction, none of the documents actually discloses the method of the invention wherein the copy number or amount of a target nucleic acid is determined, thanks to the use of a comparison to a control sequence.

4. **D1** discloses that the need of developing a method for quantification of the allele copy number based on the pyrosequencing method and that such a test was being developped.

5. The present application solves the problem by providing such a method wherein pyrosequencing is applied to a a control and a target sequence amplicon and by simply comparing the signal obtained for each amplicon.

6. The use of external controls in quantitative PCR is known from the art. **D1** for instance refers to quantitative real time PCR analysis wherein a test is carried out by amplifying KIT and a single copy control sequence (ESR).

7. However, **D1** neither gives an incentive to combine the two methods (pyrosequencing and quantitative PCR) nor any indication as to the steps that such a method should comprise.

8. It is therefore considered that the subject-matter of **claims 1-39** meets the requirements of Art. 33(2) concerning novelty and of Art. 33 (3) PCT concerning inventive step.